Supplementary Figure 1. **(a)** Brefeldin A treated (+BFA) (10μg/ml) and untreated (control) suspended cells were replated on FN (10μg/ml) for 15min, then surface labeled with CTxB-Alexa 594 (GM1-CTxB). Cells were then fixed and stained for GM130-FITC. **(b)** WT MEFs expressing GFP-tagged PKD-KD mutant (P155/287G) were surface labeled with CTxB-Alexa 594, detached and suspended for 90 min to allow endocytosis, then replated on FN (10μg/ml) for 30min. Untransfected cells marked by asterix. **(c)** Unlabelled cells expressing GFP-tagged PKD-KD and PKD-KD mutant (P155/287G) were replated on FN and surface labeled with CTxB-Alexa 594 **(d)** WT MEFs expressing GFP-tagged temperature sensitive VSV construct (VSV-GFP) + GST-PKD-KD mutant (K618N) or + GST-PKD-KD mutant (P155/287G) were sequentially incubated at 40°C, 25°C and 37°C for 15min each. Cells were fixed and stained for GST to detect PKD constructs. **(e)** Cells expressing VSV-GFP were kept at 40°C for 60min, then surface labeled with CTxB-Alexa 594 and suspended for 60min at 25°C. Lower panel **-** enlarged central region with stepwise increases in VSV-GFP intensity (green) with CTxB intensity (red) held constant. **(f)** WTMEFS labeled with Tf-Alexa 594 as above, surface labeled with CTxB-Alexa 488 on ice, suspended for 90min and replated on FN (10μg/ml) for 30min. Graph: intensities of Tf-Alexa 594 (red) and CTxB -Alexa488 (green) were measured along dotted the lines (1 and 2).

Supplementary Figure 2. **(a)** WTMEFS expressing GFP-Rab11 WT or S25N were detached, held in suspension for 90min, replated for 15min on fibronectin (2 μg/ml) and surface labeled with CTxB-Alexa594. **(b)** WTMEFS labeled with Tf-Alexa 594 were

suspended for 90min, replated on FN (10μg/ml) for 30min and area occupied by transferring determined as described in methods (n=7). **(c)** WT MEFs expressing GFP-Rab22a WT or S19N were surface labeled with CTxB-Alexa 594 (GM1-CTxB) on ice, detached and held in suspension for 90min. **(d)** Cells were replated for 15min on fibronectin (2 μg/ml). Graph: surface areas were measured; values are means ± S.E in arbitary units. (n=52 cells). Figure is best of two independent experiments.**(e)** WTMEFs expressing HA-tagged WTArf6 (WT) or Arf6T27N (T27N) were suspended for 90min and replated on FN (2 μg/ml) for 45min. Cells were then chilled, surface labeled with CTBx-Alexa 594, and images taken. Graph: surface areas were then measured; values are mean ± S.E in arbitary units. (n=25 cells). Figure is representative of two independent experiments. **(f)** Suspended vs. replated cells were surface labeled with CTxB and bound CTxB detected by Western blotting, using tubulin as a loading control. Bands were quantified, values are means ± SE, n=3, normalized for tubulin. **(g)** WT MEFs expressing HA tagged WT Arf6 or T27Arf6 were held in suspension for 90min then replated on FN at the indicated concentrations for 10min. Graph: adherent cells were counted; values are means ± SE (n=2).

Supplementary Figure 3. **(a)** Cav1$^{-/-}$ MEFs untransfected (CONTROL) or expressing WT Cav1 or Y14F Cav1, with or without HA-tagged T27N Arf6. were detached, held in suspension for 90min, replated for 15min on fibronectin (2 μg/ml), fixed and images recorded. Graph: surface areas were measured; values are mean ± S.E in arbitary units. (n=52 cells). **(b)** Adherent cells were lysed and lysates probed for caveolin (WB:Cav1), HA (WB: HA-Arf6) and tubulin (WB :tubulin). **(c)** Replated cells were surface labeled with CTxB and bound CTxB detected by Western blotting, using tubulin as a loading control. Bands were quantified, values (means ± SE, n=3, normalized for tubulin) were calculated relative to untransfected (CON) Cav1$^{-/-}$ MEFs. **(d)** WT and Cav1$^{-/-}$ MEFs expressing Arf6 (KD) or scrambled (CON) shRNA were lysed and Arf6 (WB:Arf6), Caveolin1 (WB :Cav1) and tubulin (WB:tubulin) detected by Western blotting. Figure representative of four independent experiments. **(e)** WTMEF KD and CON cell lysate were probed for Arf1, Arf3 and tubulin. Figure representative of two independent experiments. **(f)** WT MEFs expressing expressing Arf6 (KD) or scrambled (CON) shRNA

were held in suspension for 90min (SUS), replated on FN (2μg/ml) for 15min (FN 15') and incubated on ice with purified aerolysin for 30min. Bound aerolysin detected in cell lysate by Western blotting using tubulin as loading control. Bands were quantified; values are means ± SE, n=3, normalized to tubulin.

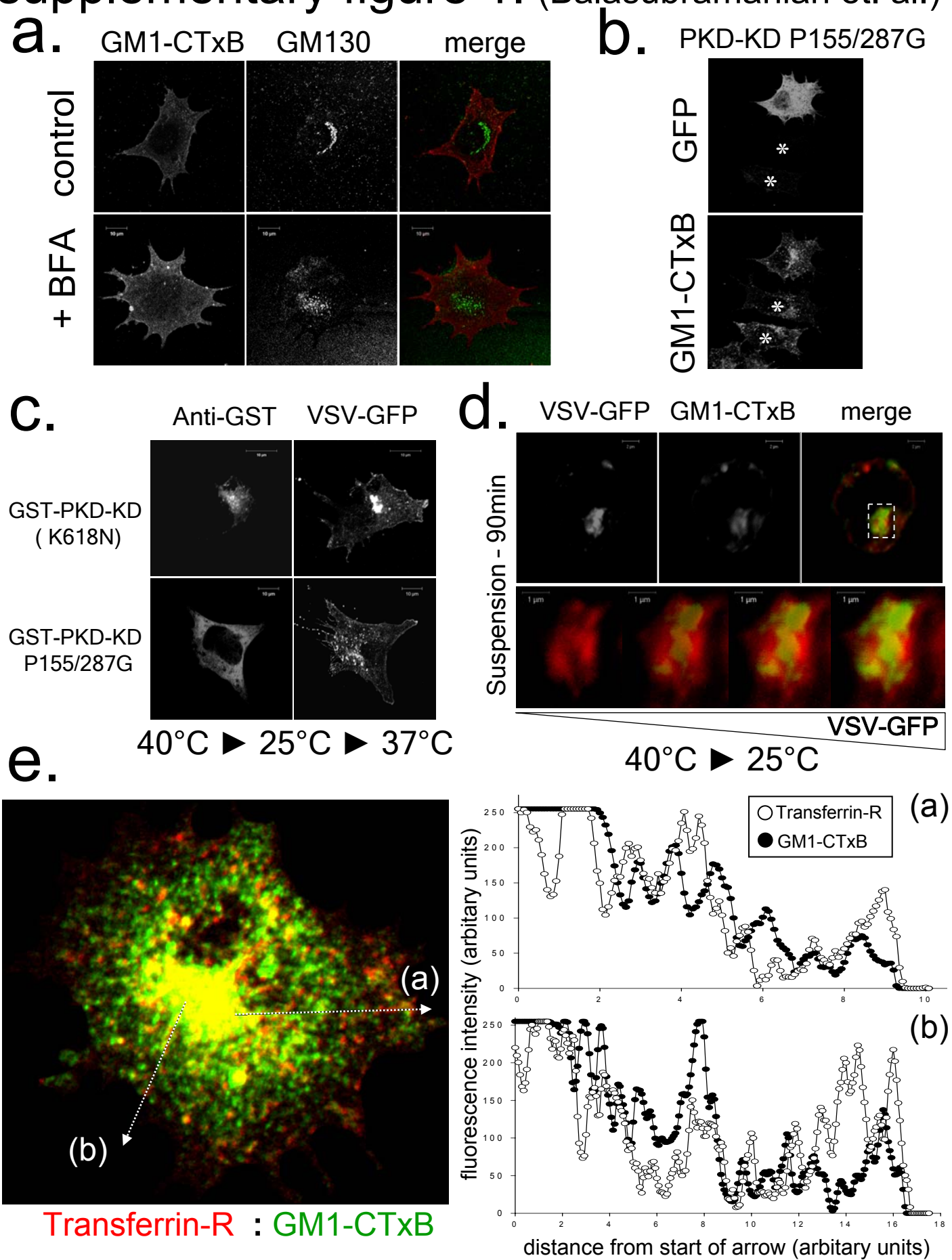
***Supplementary Figure 4*. (a)** Cav1$^{-/-}$MEFs that were stably adherent (SA), suspended for 90min (SUS), or replated on FN (10 μg/ml) for 15 or 30min (FN15', FN30') were lysed and active Arf6 pulled down using GST-GGA3 beads (GGA PD). GGA PDs and respective whole cell lysates (WCL) were Western blotted for Arf6 (WB: ARF6). Graph: bands were quantified and normalized intensities represented relative to SA. Values are means ± SE, n=3. **(b)** WT MEFs untransfected (CONTROL) or expressing T157A Arf6 (T157A) were labeled with CTxB-Alexa 594, detached, held in suspension for 90min (susp), replated on fibronectin (2 μg/ml) for 15min (FN 15'), fixed and images taken. Graph : cell surface area mean ± SE (n=75 cells). Representative of two experiments. **(c)** Cells were lysed and fractionated, and Rac1 in the membrane fraction determined as described in Methods. Graph: values are means ± SE in arbitary units, n=3. **(d)** Active Rac1 was measured using the GST-PBD pull down assay and calculated relative to total Rac1. Values are means ± SE, n=3. **(e)** Cells expressing WT Arf6 (WT) or active Arf6 (T157A) were surface labeled with CTxB-Alexa 488, detached and held in suspension for the indicated times. Representative cells in upper panel. Fluorescence intensity (as percentage of total intensity) was determined within the cell edge (●) and the cell center (□). WT Arf6 = black; T157A Arf6 = red. Values are means ± SE , n=8 cells; two independent experiments gave similar results.

***Supplementary Figure 5*. (a)** WTMEFs expressing Rab11-GFP were labeled with CTxB-Alexa 594 and left untreated (CONTROL), treated with nocodazole (10μm) before detachment (EARLY NOC) or after 90min suspension (LATE NOC). Graph : Pearson's coefficient of colocalization determined as in Methods. Values are mean ± SE, n=6. **(b)** WTMEFs were similarly untreated (control), treated early (Early NOC) or late (Late NOC) with nocodazole (10μm) . Surface GM1 was labeled with CTxB before (0'susp) or after being held in suspension (90' susp), detected by Western blotting and normalized

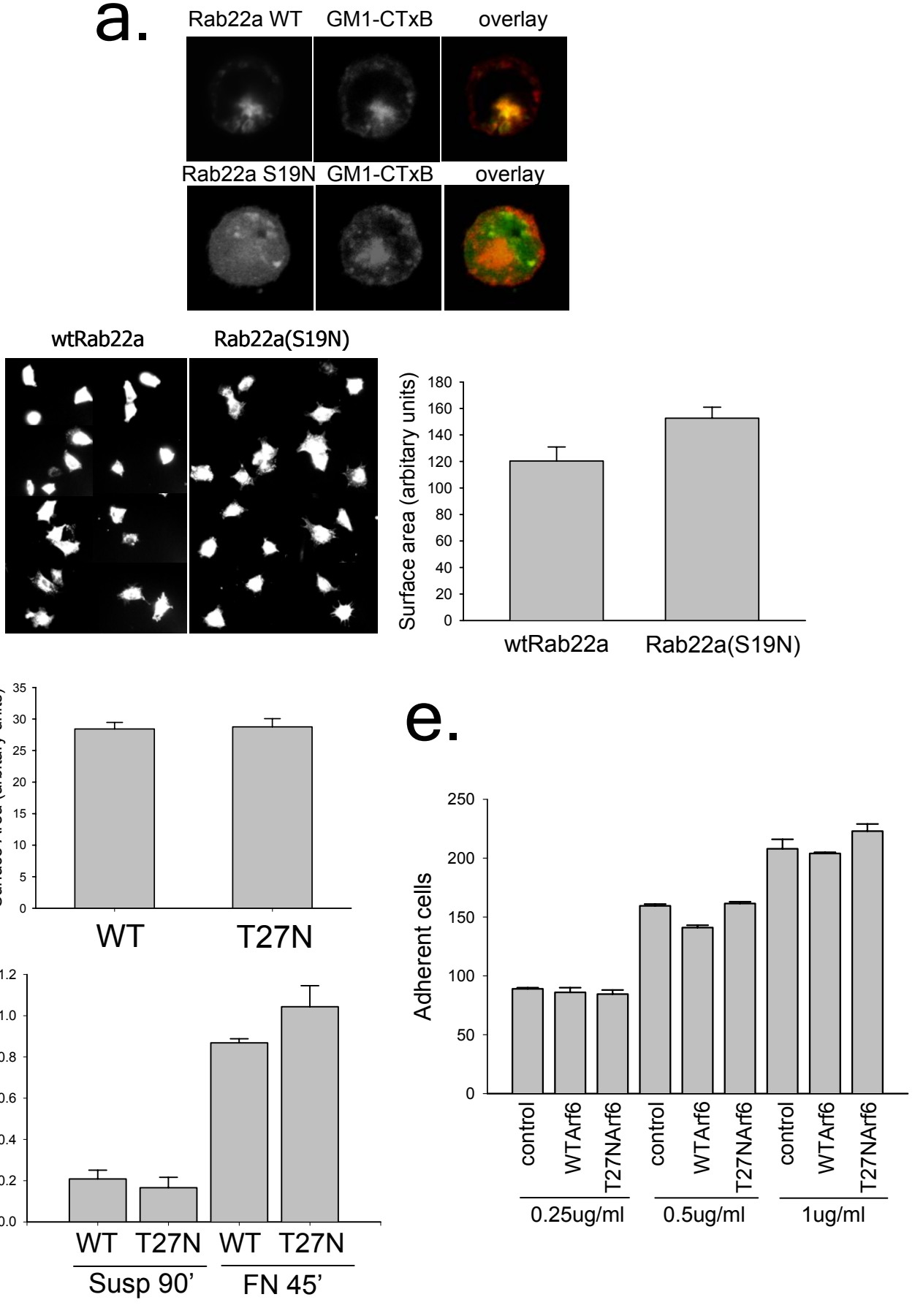
to tubulin. Graph : values are means ± SE, n=2. **(c)** WT and Cav1$^{-/-}$ MEFs were treated as above and replated on fibronectin (2μg/ml) for 15min. Cells were lysed and Rac1 in the membrane fraction determined as described in Methods. Values are means ± SE in arbitary units, n=3. **(d)** Active Rac1 was measured using the GST-PBD pull down assay and calculated relative to total Rac1. Values are means ± SE, n=3.

Supplementary Figure 6 **(a)** WT MEFs labeled with CTxB-Alexa 594, detached, held in suspension for 90 min, replated on FN (2μg/ml) for 15min, fixed and endogenous Cav-1 stained. Graph: intensities of CTxB-Alexa 594 (red) and caveolin-1 (green) along the dotted lines (1 and 2).

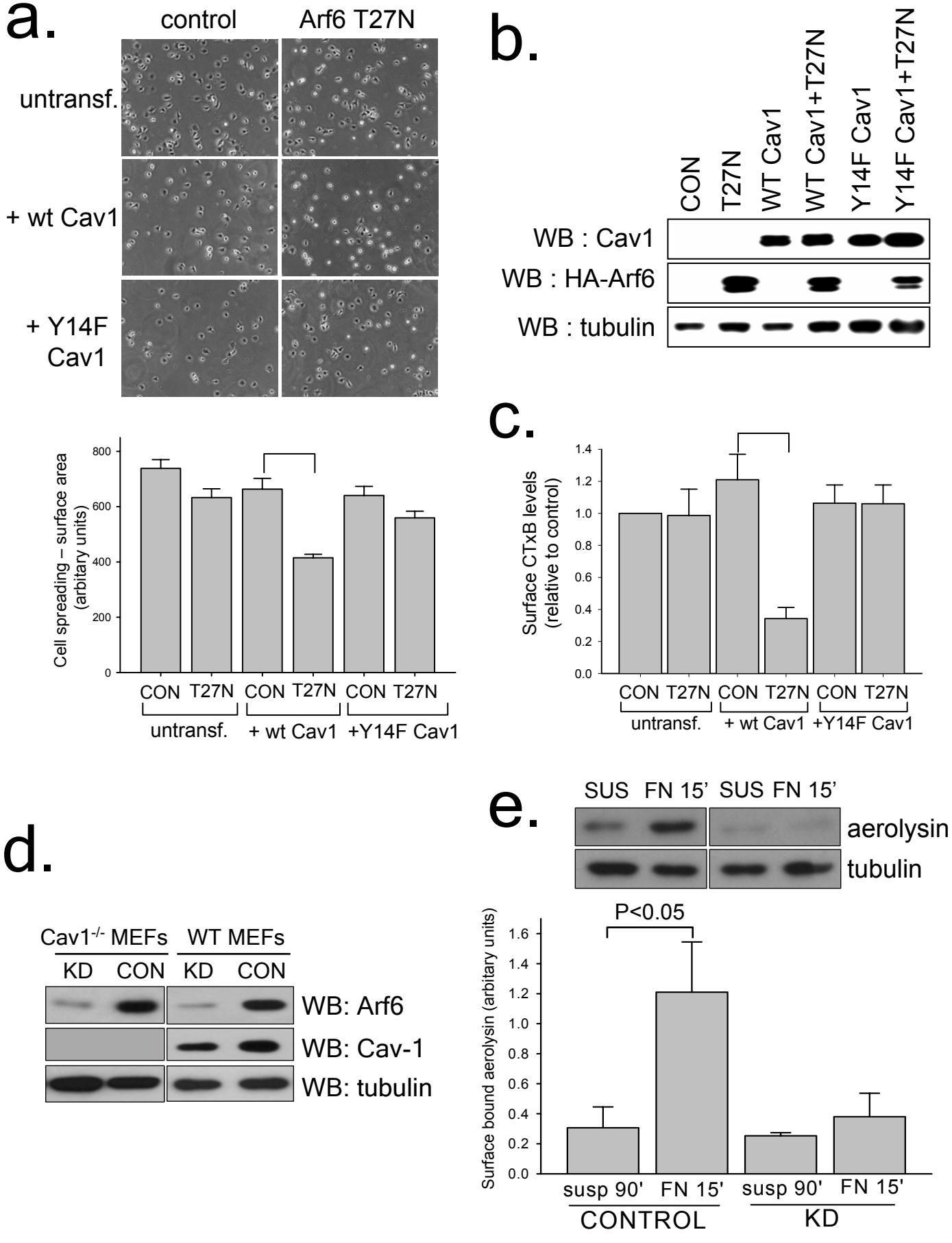
supplementary figure 1. (Balasubramanian et. al.)



supplementary figure 2. (Balasubramanian et. al.)



supplementary figure 3. (Balasubramanian et. al.)



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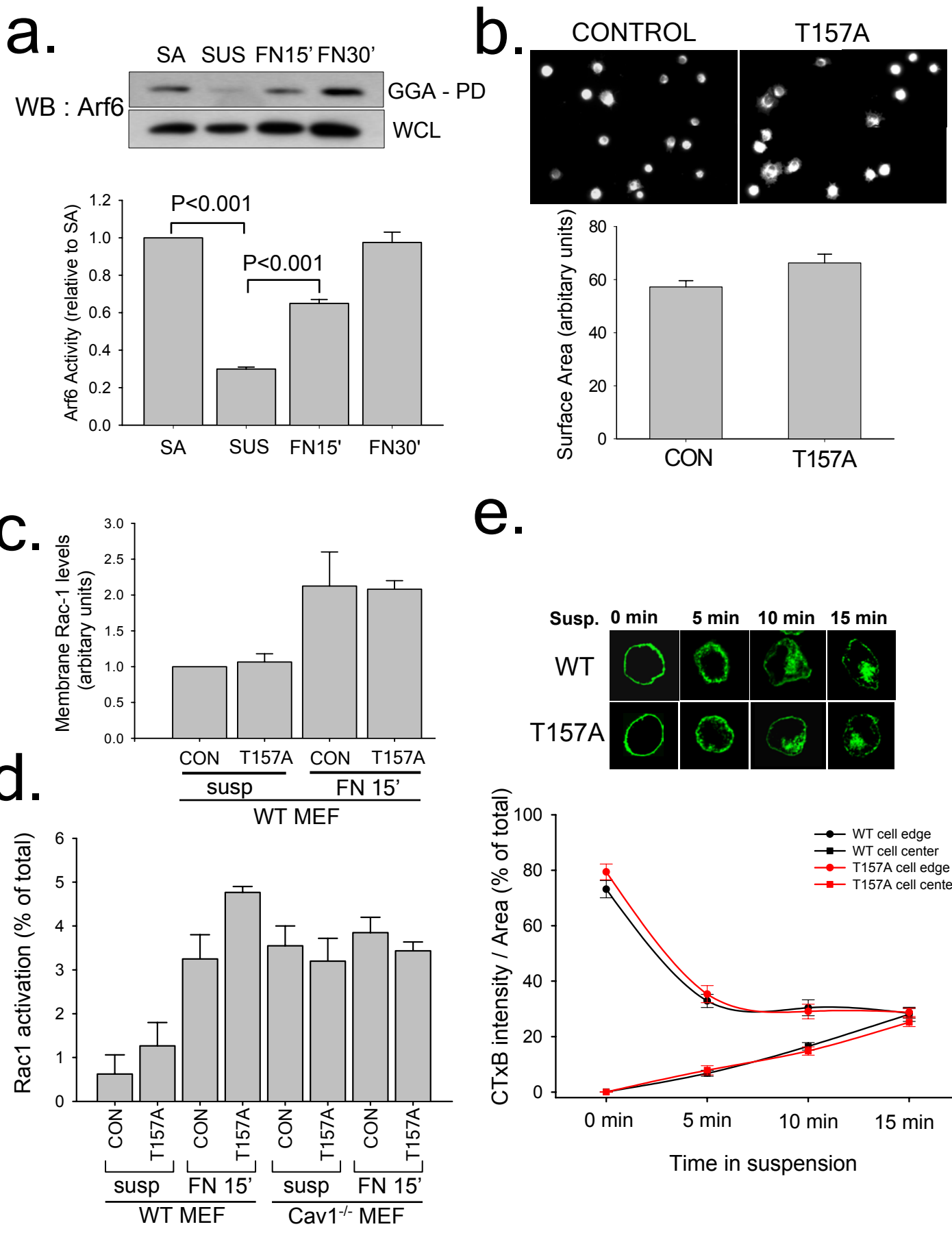
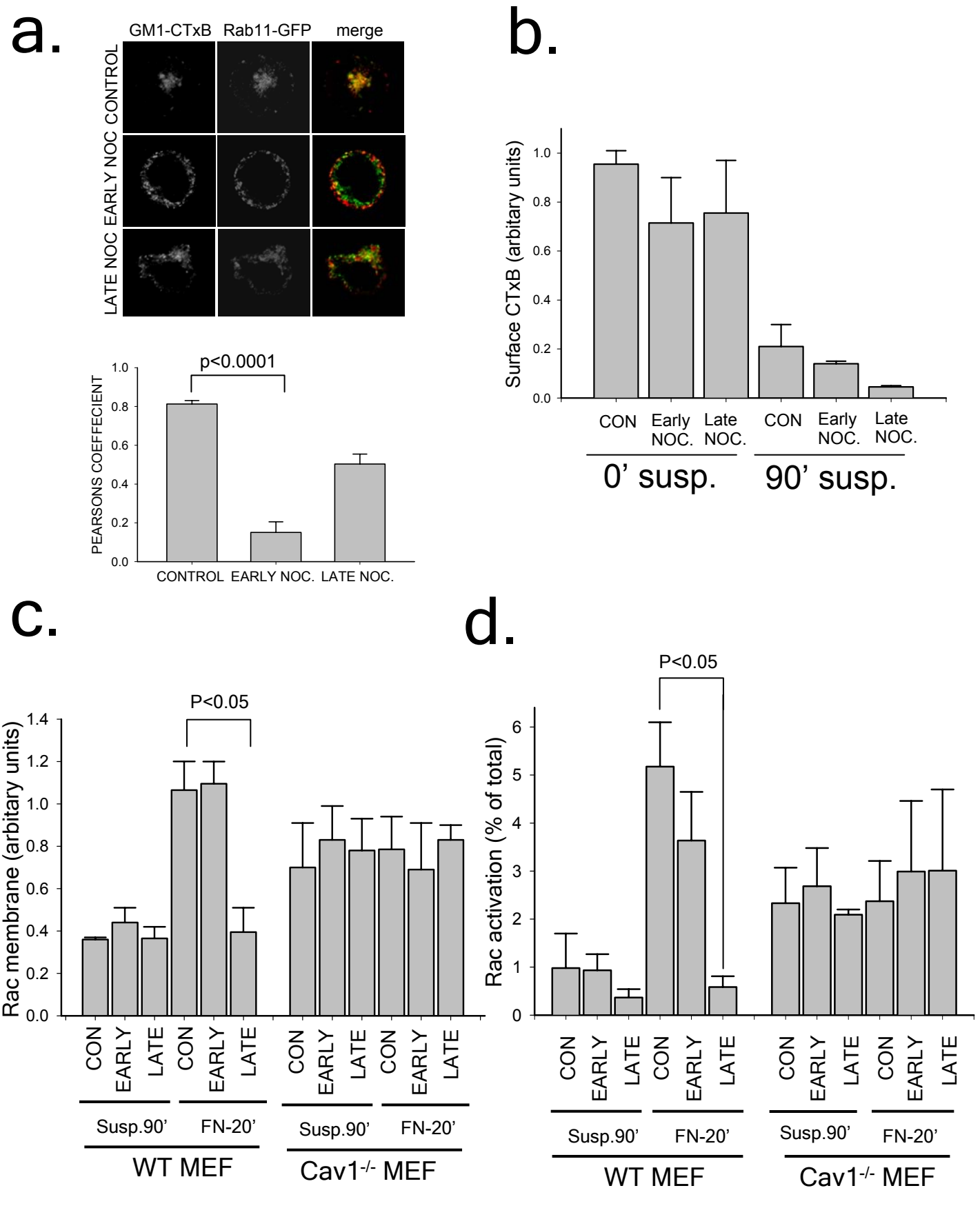
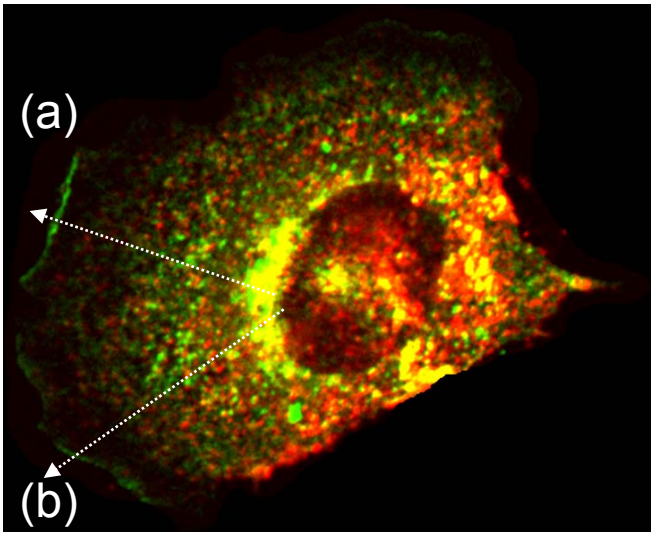


supplementary figure 5. (Balasubramanian et. al.)



supplementary figure 6. (Balasubramanian et. al.)

a.



GM1-CTxB : caveolin-1